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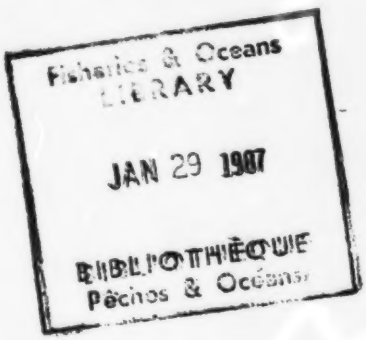

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MUCOUS AND MUCOID CELLS IN THE GASTRIC EPITHELIUM OF FISHES

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The superficial gastric epithelium shows considerable uniformity from a histomorphological standpoint over the entire range of vertebrates. It consists of a single layer of generally anisodiametric cells, in which the mucous mass is located in the supanuclear area of the cell to form the so-called "mucous plug". These characteristics are maintained, though with slight modifications as regards mainly the amount of mucus, both at the apex of the folds and at the bottom of the fossae.

Less constant is the presence of other cells, these too typically secreting mucus, which are at the point of entry of the gastric glandular tube into the gastric fossae: "the neck cells".

The neck cells, irregularly present in some species, constant in others, are described histomorphologically as large cells in which the cytoplasm almost completely filled with mucus, and having a spongy appearance, unlike the glandular appearance of the mucus of the mucous plug.

Despite the above-described histomorphological uniformity of these formations, both in the superficial mucus and in that of the neck cells, the contribution of histochemical data has enabled us to detect differences based on the composition of the mucus.

In fact, earlier authors, based on color reactions, had already distinguished from the mucous cells, other cells which they called mucoid cells, but it is to Bignardi that we owe the distinction between mucous and mucoid elements based on histochemical characters. Bignardi, in making his distinction, confirmed the metachromasia reaction, which was then proposed by Lison to demonstrate the sulphuric esters of mucopolysaccharides. Beginning with Bignardi's research, the distinction was somewhat unclear, insofar as metachromasia may occur also under other conditions, and does not always indicate the presence of sulphuric esters of mucopolysaccharides. However, the breakdown of the muciparous elements into elements with positive metachromasia (mucous) and elements with negative metachromasia (mucoid) has represented and can still represent a distinguishing character based on histochemical criteria.

It is useless to delve into much debated questions which are not yet resolved on the significance of metachromasia; rather, it is sufficient to state that mucoid cells are not defined solely by a negative finding (absence of metachromasia), but also by something that they have in common with the mucous cells, namely their response to the Bauer reaction and hence to the various oxidation reactions later added to it. There is also no need to discuss here the other histochemical characteristics which serve to define the various types of muciparous cells.

The distinction between mucous elements and mucoid elements is not however very clear and there is a whole gamut of cell types which combine these two extreme forms. Because of the difficulty of defining a clear limit, Zanotti proposed a category of elements between mucous and mucoid, to which he has given the name of transition cells.

This was the state of existing knowledge when studies of comparative anatomy were carried out by Zanotti and Camisani in our Institute, on mammals and amphibians respectively.

In the long time which has elapsed since the works of Zanotti and Camisani, new technical possibilities have presented themselves, which we need to take into account in extending the research to other groups of vertebrates. These possibilities include the generic study of the mucopolysaccharides, for which, to the methods using chromic oxidation, are added those using potassium permanganate, periodate, lead tetracetate and sodium bismuthate as oxidizing agents; and the argentaffin techniques to detect aldehydes formed during the oxidation process. Also, as regards the more or less acid nature of the mucus with the metachromasia reaction, we now have the Hale reaction with colloidal iron hydrate and staining with Alcian blue 8 GN demonstrated by Vialli and corresponding to the metachromasia reaction. Recently there has been a refinement of the Hale-Hotchkiss and Alcan-Hotchkiss double reactions, which permit diagnostic positivity of the respective methods used individually, nor should it be forgotten that oxidizing interventions, and in particular that using acid, can bring about reactions characteristic of mucous cells in mucoid cells.

This whole complex of reactions apparently has not been used up to now in comparative anatomy studies on gastric mucus in the

different groups of vertebrates. Only very recently has the question been taken up again in our Institute, by myself for fish, by Fantin for birds and by Spallazzi for the muciparous cells of esophagus and stomach of frog.

For this research, I studied fragments of the intermediate part of the stomach of the following 17 species of fishes:

Selachii: Scyliorhinus stellaris L.; Torpedo marmorata Risso; Torpedo ocellata L.

Chondrostelon ganoidel: Acipenser sturio L.; Acipenser naccarii Bp;

Teleostei: Amiurus catus L.; Anguilla vulgaris Turt.; Esox lucius L.; Perca fluviatilis L.; Grystes salmoides GÜnth; Serranus hepatus L.; Serranus cabrilla L.; Boops salpa L.; Sargus annularis Gmelin; Sargus rondeleti Cuv. and Val.; Phterophyllum scalare Licht; Scorpaena porcus L.

The freshly collected material was fixed in 10% formalin for a period of 8 days, washed in water and enclosed in paraffin in the usual manner. I used the Hotchkiss reaction and the Bauer reaction according to the standard method. For the metachromasia reaction, I used the same method already employed by Zanotti and Camisani; (1% Toluidine blue for 3 to 5 minutes, the sections were observed in water and subsequently in glycerin noting the result after a few minutes and after 24 hours). In the same way, I used the metachromasia reaction on material chromized according to Bignardi, (10% chromic acid for one hour, washing at length also in alcohol at 70°, prior to staining with Toluidine blue).

For the Hale reaction, I used colloidal iron hydrate prepared according to the technique suggested by MÜller, while for the rest I followed what is normally recommended in the literature.

The simultaneous reactions with Alcian blue 8 GN-Hotchkiss and Hale-Hotchkiss were obtained in the sequence indicated.

I already made mention of the fact that neck cells are present only in some species of fish; for this reason, I was always concerned with seeing whether or not they could be detected. The question is of definite importance in relation to the data of Barrington, who also cites previous observations by Bensley and Plenck.

So as not to waste time discussing data that very often are quite similar, I shall limit myself to describing in detail the histochemical picture observed in the first species on the list in each case, indicating that the others follow more or less the same pattern.

Scyliorhinus stellaris. The Hotchkiss reaction and to a lesser extent the Bauer reaction, are positive in the mucus both at the apex of the folds and in the bottom of the gastric fossae, the mucous plug is densely filled with granules all reacting with equal intensity. When stained with Toluidine blue under the conditions described above, the mucous plug appears bleached, after chromization there is a well-defined pink metachromasia, which persists in glycerin for a short time, but is almost completely eliminated 24 hours after mounting in glycerin. Following staining with Alcian blue, on the other hand, the mucus is moderately colored both at the apex of the crests and at the bottom of the folds. Chromization, though reducing the general stainability of the preparation, does not specifically affect stainability of the mucus with Alcian blue. Less clear is the response

Fig. 1. Torpedo ocellata. Stomach-Hotchkiss reaction 200 x

Fig. 3. Serranus cabrilla. Hotch-kiss reaction. 200 x

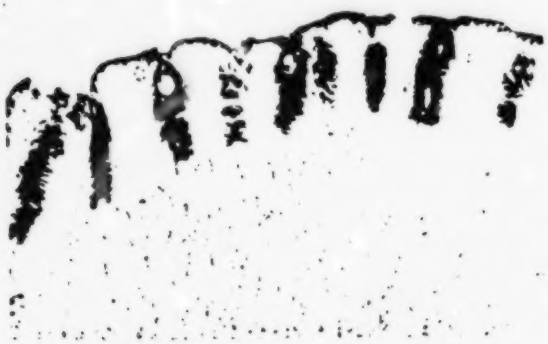

Fig. 2. Esox lucius. Stomach - Hotchkiss reaction. 200 x

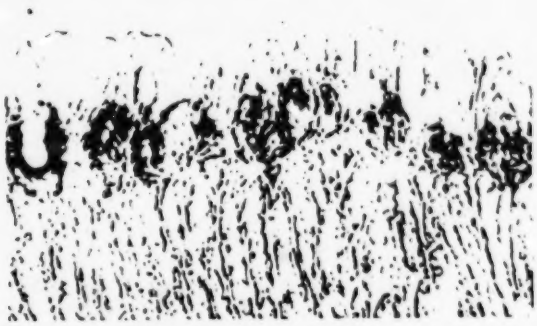

Fig. 4. Serranus cabrilla. Stomach-Hale reaction. 264 x

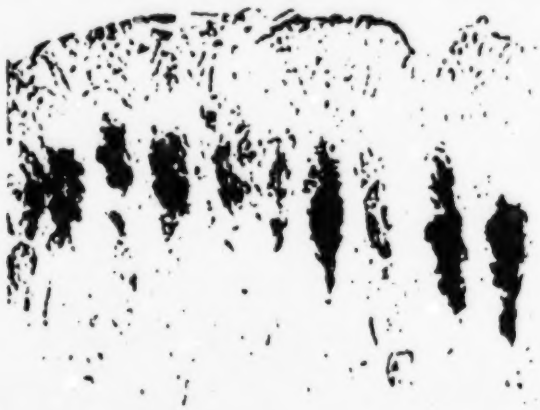

Fig. 5. Serranus cabrilla. Stomach. Staining with Alcian blue 8 GN. 264 x

of the Hale reaction, where one notes a certain positivity of the mucous plug without it being possible to distinguish individual granules. The double reaction with Alcian blue - Hotchkiss gives the mucus a reddish-violet coloration due to the simultaneous response of the two reactions. With the Hale-Hotchkiss reaction, on the other hand, there is a slight predominance of the red. Overall, the mucus of this species cannot be called typically mucoid, but should be defined as being of the transition type.

The same conditions obtain for Torpedo ocellata and Torpedo marmorata.

For both Acipenser sturio and for Acipenser naccarii, the pictures are similar. The Hotchkiss reaction and the Bauer reaction are positive, with high color intensity both in the epithelium of the folds and along the walls of same. Mucus reactivity diminishes however at the bottom of the gastric fossae where there is less intense coloration of the granules; they appear to be fewer in number. Upon observation, the metachromasia reaction reveals the presence, at the bottom of the gastric fossae, of metachromatic mucus, which disappears in glycerin, while the mucous plug of the remaining parts of the epithelium is typically bleached. Chromization causes pinnk metachromasia in the mucous plug at all levels and does not enable one to detect differences. These are very evident, on the other hand, in both the Hale and the Alcian reactions, which are positive only in the deepest cells of the gastric fossae, and in double Alcian-Hotchkiss and Hale-Hotchkiss staining where, along the length of a tubule, one notes the blue colour of the mucous plug of the deepest cells and the red colour of the superficial cells. Observation of cross sections in

tubules in some cases enables one to see cells of varying degrees of stainability mixed with extremes showing typically positive Hotchkiss reactions and typically positive Alcian reactions.

Amiurus catus. The Hotchkiss and Bauer pictures are clearly positive for the mucous plug (in my preparations no neck cells were noted in this species). Metachromasia is negative, while chromization causes a certain not very persistent pink coloration of the mucous plug. Staining with Alcian and the Hale reaction both give negative results.

This is obviously a mucoid location.

The histochemical picture offered by Amiurus catus is repeated, generally speaking, in the following species: Anguilla vulgaris, Esox lucius, Perca fluvialis, Grystes salmoides, Sargus annularis, Sargus rondeleti and Ptherophyllum scalare.

Serranus hepatus. In this species, neck cells are clearly present at the entry of the glandular tubule into the bottom of the gastric fossa, are quite numerous with 4 or 5 in each longitudinal section and show a characteristic behaviour. The Hotchkiss and Bauer reactions do not give coloured reactions for the neck cells, while they are markedly positive in the mucous plug. Metachromasia, on the contrary, while it is negative in the mucous plug, which it leaves bleached, causes the neck cells to take on a pink colour which persists quite well in glycerin. After chromization, a difference can still be noted in that the neck cell material is more coloured than that of the mucus plug, in which a pink metachromasia also appeared. The difference in behaviour between the two locations is also demonstrated by the Alcian and Hale reactions, which are positive in the neck cells. This picture also appears very clearly with Alcian-Hotchkiss and

Hale-Hotchkiss double staining, with a clean prevalence of the Alcian and the Hale. It is evident that the neck cells can be considered to be typically mucous in character, in contrast with the mucoid character of the mucous plug of the superficial epithelium, which does not differ very much in this regard from the corresponding location in the other species of teleosts observed.

Serranus cabrilla and Scorpaena poreus behave like Serranus hepatus, both as regards the neck cells and as regards the superior mucous plug.

Boops salpa. In this species, in which neck cells cannot be detected, at least not in my preparations, the Hotchkiss and Bauer reactions are intensely positive in all cells of the mucous plug. Staining with Toluidine blue results in intense metachromatic coloration, which persists in glycerin and is therefore such as to be considered an orthodox metachromasia. Staining with Alcian also gives a positive though not very strong reaction. The Hale reaction gives a weak response. Double staining with Alcian-Hotchkiss shows unequivocally (blue-dark reddish coloration) that in the mucous plug there is a simultaneous response by both reactions. The response of the Hale-Hotchkiss on the other hand indicates a prevalence in the mucous plug of the Hotchkiss reaction. In this species, therefore, the mucus located in the mucous plug is more acid than we observed in the other species and enables us to define the epithelial cell as a mucous cell.

CONCLUSIONS

The examination of the histochemical behaviour of the mucus in species of selachians, ganoids and teleosts, though limited for now

to a comparison of a limited number of species, and therefore needing to be extended, already indicates a certain number of differential characteristics in the overall material considered.

In the selachians, we observed a mucus of a transitional nature between mucoid and mucous, with some differences in the degree of referability to one or the other type. However, among those observed, we in no instance found differences in response between cells at the apex of the folds and at the bottom of the fossae.

In the two species of ganoids observed, the mucus of the superficial cells at the apex of the gastric folds is typically mucoid; it is interesting to note, however, that the mucus of the deeper cells, in the fossae, assumes a more acid character (definable as mucous). In the transition zone, it is possible to note mucous cells and mucoid cells adjacent or even mixed together at some points.

In all the species of teleosts observed, except for Boops salpa the mucous plug of the superficial epithelium is mucoid, with no differentiation between the bottom of the fossae and the apex of the folds. It should be pointed out that in Serranus hepatus, in Serranus cabrilla and in Scorpaena procus, that is to say, in the only species studied by me which showed visible neck cells in my preparations, these have a typically mucous character, contrasting therefore with those of the superficial mucus.

The presence of a single species in which the superficial epithelial cells are mucous cells suggests the need to extend this study to other species, in a research which takes into account the different functional moments to determine whether this is a constant or

a sporadic condition. It is interesting that all the superficial muciparous cells are mucoid in the species of the various groups considered. This fact is consistent with the similar finding of Camisani in amphibians, while it contrasts with the variability of conditions from species to species observed by Zanotti in mammals and by Fantin in birds, a fact which, even in the absence of similar research on reptiles can, at least by way of hypothesis, be considered linked to greater gastric secretion by warm-blooded animals.

The topographical arrangement, in which the cells of the superficial epithalia of the acipenseroids are mucous in the deeper parts of the gastric fossae as opposed to those of the apices which are mucoid, finds support in the fact that the neck cells present in Serranus hepatus, Serranus cabrilla and in Scorpaena porcus show mucous secretion while the superficial epithelium shows mucoid secretion. One could draw conclusions from this arrangement, which however it seems advisable to delay until such time as more comparative research has been conducted also because it will enable us to see to what extent these data may be generalized, since Faulin's results on birds, for example, show the reverse of this situation.

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